

## **MARKED-UP VERSION OF AMENDED PARAGRAPHS IN THE SPECIFICATION**

In the following amendments, underlining denotes added text while bracketing denotes deleted text. For the Tables, the only changes involve deleting the original shading within some cells and replacing it with text boxes. As these changes are too difficult to show with underlining and bracketing, no underlining or bracketing are used below. Should the Examiner require a different format to indicate these amendments, she is encouraged to contact Applicants.

### **IN THE SPECIFICATION:**

Please replace the paragraph on page 1 under "Cross-Reference to Related Applications" with the following:

Pursuant to 35 U.S.C. §119(e), the present application claims benefit of and priority to US Application No. 60/233,610, entitled "Twin-Arginine Translocation in Bacillus", filed September 18, 2000<sup>[1]</sup> by Jongbloed et al.

Please replace Table I and the text following the Table, on page 56 with the following:

**Table I. Predicted Twin-Arginine Signal Peptides of *B. subtilis*\***

<b>protein</b>	<b>signal peptide</b>
AlbB	SPAQRRIILYILSFIFVIGAVVYFVKSDYLFTLIFI <sup>1</sup> AIAILF
AmyX <sup>TM</sup>	MVSIRRSFEAYVDDMNIIITVLIPA <sup>2</sup> EQKEIM
AppB <sup>TM</sup>	MAAYIIIRRT <sup>1</sup> LMSIPILLGITILSFVIM <sup>2</sup> KAAPG
LipA	MKFVKRR <sup>1</sup> IIALVTILMLSVTSLFAL <sup>2</sup> QPSAKAAEH
OppB <sup>TM</sup>	MLKYIGRR <sup>1</sup> LVYMIITLFVIVTVTFFLM <sup>2</sup> QAAPG
PbpX	MTSPTRRRTAKRRRRKLNKR <sup>1</sup> GKLLFGLLAMVCITII <sup>2</sup> WNALHR
PhoD	MAYDSRFDEWVQKLKEESFQNNTFDRRKFI <sup>1</sup> QGAGKIAGLSLGLTIAQS <sup>2</sup> VGAFEV
QcrA	MGGKHDISRQFLN <sup>1</sup> YTLTGVGGFMAASMLMPMV <sup>2</sup> RFA
SpoIIIJ <sup>TM</sup>	MLLKRR <sup>1</sup> IGLLLSMVGVFML <sup>2</sup> LAGCSSV
TlpA <sup>TM</sup>	MKKTLTITRRSSIARR <sup>1</sup> LIISFLLILIVPITALSVSAY <sup>2</sup> QS
WapA	MKKRKRNFKR <sup>1</sup> FIAAFLVLALMISLVPAD <sup>2</sup> DVLAKST
WprA	KRRKFSS <sup>1</sup> VVAAVLIFALIFSLFSPG <sup>2</sup> TKAAAAGA
YceA <sup>TM</sup>	MEMFDLEFMRR <sup>1</sup> AFLAGGMIAVMAPILGVYLVL <sup>2</sup> RRQ
YdeJ	MKKRRK <sup>1</sup> ICYCNTALLMII <sup>2</sup> LAGCTDS
YdhF	MRR <sup>1</sup> ILSILVFAIM <sup>2</sup> LAGCSSN
YdhK	MSAGKSYRKKMKQRRMNMKISK <sup>1</sup> YALGILMLSLVFV <sup>2</sup> LSACGNNN
YesM <sup>TM</sup>	KKRVA GWYRRMKIKDK <sup>1</sup> LFVFLSLIMAVSFLFVYSGV <sup>2</sup> QYAFHV
YesW	MRRSCLMIRRRKR <sup>1</sup> MFTAVTLLVLLVMGTSVCP <sup>2</sup> VKAEGA
YfkN <sup>TM</sup>	MRIQKRRTHTVENILR <sup>1</sup> ILLPPIMILSLILPTPP <sup>2</sup> IHAES
YkpC	MLRDLGRR <sup>1</sup> VAIAAILSGIILGGMSI <sup>2</sup> SLANMP
YkuE	MKKMSRRQFLK <sup>1</sup> GMFGALAAGALTAGGGY <sup>2</sup> GYARYL
YmaC	MRRFLN <sup>1</sup> VILVLAIVLFLRYV <sup>2</sup> HYSLEPE
YmzC	MFESAEELRR <sup>1</sup> IRIALVWIAVFL <sup>2</sup> LFGACGN
YubF <sup>TM</sup>	MQKYRRRNT <sup>1</sup> VAFTVLAYFTFFAGVFLFSIGLY <sup>2</sup> NADNL
YuiC	MMLNMIRRL <sup>1</sup> LMTCFLFLAFGTTFLSVSG <sup>2</sup> IEAKDL
YvhJ	MAERVVRVRKKKKSKRRKILKR <sup>1</sup> IMLLFALALLVVVGLGGY <sup>2</sup> KLY
YwbN	MSDEQKKPEQIHRRDILK <sup>1</sup> WGAMAGAAVAIGASGLGGLAPLV <sup>2</sup> QTAAPK

\* Putative twin-arginine signal peptides were identified in two ways. First, the presence of the

consensus sequence R-R-X- $\phi$ - $\phi$  ( $\phi$  is a hydrophobic residue), immediately in front of an amino-terminal hydrophobic region as predicted with the TopPred2 algorithm (34, 35), was determined. To this purpose, the first 60 residues of all annotated proteins of *B. subtilis* in the SubtiList database (<http://bioweb.pasteur.fr/Genolist/Subtilist.html>) were used. Second, within the group of twin-arginine membrane sorting signals, cleavable signal peptides were identified with the SignalP algorithm (61, 62). Conserved residues of the twin-arginine consensus sequence (R-R-X- $\phi$ - $\phi$ ) are indicated in bold. In addition, positively charged residues that could function as a so-called Sec-avoidance signal (54) are indicated in bold and italics. The hydrophobic H-domain is indicated in gray shading in boxed text. In signal peptides with a predicted signal peptidase I cleavage site, residues from position -3 to -1 relative to the signal peptidase I cleavage site are underlined. Notably, some of these proteins contain one or more putative transmembrane segments elsewhere in the protein (indicated with "TM"), or are putative lipoproteins. Residues forming a so-called lipobox for signal peptidase II cleavage are enlarged in size.

Please replace Table IV and following text, on page 59, with the following:

**Table IV. Twin-Arginine Signal Peptides of PhoD and PhoD-like proteins\***

protein	signal peptide
PhoD (Bsu)	MAYDSRFDEWVQKLKEESFQNNTFDRRKFIQ <b>GAGKIAGLSLGLTIAQS</b> VGAFEV
SP1 (Sco)	MTPANHQAPTSAPSPAPSQSSHAPELRAAARSLGRRRFLT <b>VTGAAAALAF</b> AVNLPAAGTA
SP2 (Sco)	MAPTGRPSALAEHAFSPHDAVLGAAARHLGRRRFLT <b>VTAAAAALAF</b> STNLPARGAVAAPE
SP3 (Sco)	MTSRHRASENSRTPSRRTVVK <b>AAAAGAVLAAPLAAALPAGA</b> ADAAPA
SP4 (Ste)	MTPAARPSQHAPELRAAARHLGRRRFLT <b>VTGAAAALAF</b> AVNLPAAGT <b>AAAAEL</b>

\* Homologues of *B. subtilis* PhoD were identified by amino acid sequence similarity searches in GenBank using the BLAST algorithm. SP1 (Sco), gene SCC75A.32c of *Streptomyces coelicolor* (CAB61732); SP2 (Sco), gene SCF43A.18 of *S. coelicolor* (CAB48905); SP3 (Sco), gene SC4G6.37 of *S. coelicolor* (CAB51460), and SP4, *phoD* gene of *Streptomyces tendae*

(CAB62565). GenBank accession numbers are indicated in parentheses. Conserved residues of the twin-arginine consensus sequence are indicated in bold. The hydrophobic H-region is indicated by boxed text. ~~in in gray shading.~~ Signal peptidase I recognition sequences predicted with the SignalP algorithm (61, 62) are underlined.